In Situ Localization of Interleukin-1 in Normal and Psoriatic Skin

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Interleukin-1 (IL-1) is a family of polypeptides that mediates a wide range of inflammatory and immune responses. In human skin, unstimulated keratinocytes produce a large amount of such cytokines. Although the precise role of IL-1 in the skin is unknown, there is experimental evidence supporting involvement of IL-1 in the pathogenesis of inflammatory skin diseases. In this study, we investigated the in situ localization of IL-1 alpha and IL-1 beta in normal and psoriatic skin. Using polyclonal antibodies and the avidin-biotin peroxidase complex, we demonstrated the presence of both IL-1 alpha and IL-1 beta in normal and psoriatic formalin-fixed paraffin-embedded tissues. In both cases, IL-1 alpha was more prominent. However, the distribution of IL-1 alpha differed between normal and psoriatic skin. In normal skin, IL-1 alpha distribution was predominantly intercellular, whereas IL-1 alpha distribution was predominantly within the cytoplasm in psoriatic skin. These studies confirm that IL-1 alpha is the predominant form of IL-1 in the skin and provide further support for the hypothesis that IL-1 participates in the pathogenesis of psoriasis. *J Invest Dermatol 93:518–522, 1989*

Within the last decade a large amount of clinical and experimental evidence has accumulated supporting the hypothesis that interleukin-1 (IL-1) plays an important role in inflammatory and immune responses [1,2]. IL-1 was originally defined by its ability to enhance lymphocyte proliferation and, later, by possession of a number of biologic activities, including induction of the acute phase response and systemic growth and differentiation signals for immune cells [1,2]. IL-1 is a potent neutrophil and T-cell chemoattractant [1,3] and has the ability to induce murine epidermal hyperplasia and subcutaneous fibrosis in vivo [4].The cloning of two distinct forms of IL-1, alpha and beta, has permitted confirmation of these biologic properties. Both forms of IL-1 share little amino acid homology and have the same spectrum of biologic activities [5,6], presumably mediated by binding to the same receptor [7].

Although phagocytic cells are the main source of IL-1 [1,2], other cellular types also produce this cytokine. In the human epidermis, both Langerhans cells and keratinocytes have been shown to produce an epidermal cell-derived thymocyte activating factor (ETAF) [8–11] with biochemical and biologic properties identical to those of human macrophage/monocyte-derived IL-1 [11,12]. Whether ETAF is structurally identical to monocyte IL-1 has not been entirely resolved [13]. Unstimulated keratinocytes produce predominantly IL-1 alpha and smaller amount of IL-1 beta as identified on Northern blots [11]. Similar results have been found in murine epidermis by in situ hybridization, suggesting that IL-1 alpha is the dominant species of IL-1 in skin [14]. IL-1 activity may also be detected in high amounts in stratum corneum and in cultured human epidermal cells [15,16]. However, the subcellular site of IL-1 production in epidermal cells has not been identified.

IL-1 acts in an autocrine manner to stimulate epidermal growth as demonstrated by stimulation of keratinocyte proliferation and DNA synthesis in cultures [17,18]. Because of these autocrine effects as well as IL-1 actions on neutrophils, a role for IL-1 in psoriasis has been sought [19,20]. Recent studies have used immunostaining and bioassays to detect IL-1 in psoriatic plaques and scales [20,21]. We have asked whether IL-1 alpha and IL-1 beta can be detected in psoriatic skin. Our studies establish an alterated distribution of IL-1 alpha and IL-1 beta in psoriatic skin and support the hypothesis that IL-1 is involved in the pathogenesis of psoriasis.

MATERIALS AND METHODS

Preparation of Specimens Twelve formalin-fixed paraffin-embedded biopsies from patients with clinical and histopathologic features consistent with psoriasis were obtained from the Pathology Department at the New England Medical Center Hospital (Table I). Twelve normal skin samples were received from the Dermatologic Surgery Department from patients undergoing plastic reconstructive surgery. These tissues were fixed in formalin as above and used as a control skin.

Antibodies Anti-human IL-1 alpha and anti-human IL-1 beta rabbit polyclonal antibodies (IgG) raised against recombinant human IL-1 alpha and beta, respectively, were produced and kindly provided by Dr. C.A. Dinarello. Rabbits were immunized with multiple intradermal injections in complete Freund's adjuvant. After 6 weeks, rabbits received four weekly i.m. injections in incomplete Freund's adjuvant and four weekly i.v. injections. Rabbits were bled, and serial dilutions of 40% ammonium sulfate precipitated globulin preparations were shown to have a neutralizing effect on the respective forms of IL-1 over a wide concentration range. They did not cross-react as determined by spectrophotometric analysis [22]. These antibodies were employed as the primary antibody at an optimal concentration of 2 μg/ml.

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Abbreviations:

ABC: Avidin-Biotin Peroxidase Complex
ETAF: Epidermal Cell-derived Thymocyte Activating Factor
IL-1: Interleukin-1
PBS: Phosphate Buffered Saline

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Table I. Patients with Psoriasis

Patient*	Age	Sex	Clinical Classification of Psoriasis
1	66	F	Pustular psoriasis
2	40	F	Psoriasis vulgaris
3	33	M	Psoriasis vulgaris
4	37	M	Psoriasis vulgaris
5	33	F	Psoriasis vulgaris
6	9	F	Psoriasis vulgaris
7	27	M	Psoriasis vulgaris
8	49	F	Psoriasis vulgaris
9	59	F	Psoriasis vulgaris
10	73	F	Psoriasis vulgaris
11	43	M	Psoriasis vulgaris
12	3	M	Psoriasis vulgaris

* Biopsies for confirmation of the clinical diagnosis of psoriasis were obtained from these patients at the New England Medical Center Hospital. All charts were reviewed to confirm that patients had both clinical and histologic features of psoriasis.

Cytochemical Visualization of Skin Tissue The avidin-biotin peroxidase technique was used for detection of IL-1. The ABC kit from Vectastain laboratories [23,24] was used as an immunoenzymatic technique. Briefly, 6-μm sections of the tissues were cut and placed on gelatin coated slides, deparaffined by xylene, and rehydrated through graduated ethanol solutions. The endogenous peroxidase was blocked with 0.9% hydrogen peroxide in phosphate buffered saline (PBS, pH 7.2) for 30 min at room temperature. After washing twice with buffer, the tissue sections were incubated with 1% normal goat serum in PBS for 60 min to avoid nonspecific binding. The excess solution was removed and the sections were incubated with 1:100 or 1:1000 dilutions of anti-human IL-1 alpha or anti-human IL-1 beta for 60 min. The slides were then washed twice with PBS 0.1% BSA and incubated with a biotinylated goat anti-rabbit IgG for 45 min. After further washing, the tissue sections were incubated with the avidin-biotin-peroxidase complex for 30 min and washed with buffer. The specific substrate (1 mg/ml diaminobenzidine tetrahydrochloride in 0.1 M tris buffer plus 0.01% hydrogen peroxide in distilled water) was added for 5 min. Finally, the sections were washed with tap water for 5 min, counterstained with methyl green, coverslipped with Aquamount, and examined under light microscope. An irrelevant rabbit IgG was included in each assay and used as a control. All of the incubations in the avidin-biotin-peroxidase technique were done in a moist chamber at room temperature.

Blocking Studies In order to test the specificity of the reaction, serial dilutions of the recombinant IL-1 alpha and IL-1 beta were pre-mixed with 2.5 μg/ml and 5 μg/ml of anti-IL-1 alpha and anti-IL-1 beta, respectively. Preincubation with a combination of 5 μg/ml of anti IL-1 beta with serial dilutions of recombinant IL-1 alpha was done in order to evaluate cross-reactivity. This mixture was left overnight at 4°c before being added to the tissues. The antibodies mixed with PBS were used as a positive control.

RESULTS

Cytochemical Visualization of Tissue IL-1 We used a three-step avidin-biotin-peroxidase technique in order to determine whether IL-1 alpha and/or IL-1 beta could be detected within normal human epidermis. There were two different but non-exclusive patterns of cellular localization of IL-1 for both IL-1 alpha and IL-1 beta (Fig 1). In many specimens, staining was most apparent around the cell membrane and is defined as intercellular. In other specimens, IL-1 appeared within the cytoplasm and is defined as a intracellular cytoplasmic distribution. Indirect immunofluorescence using the same antibody dilutions also confirmed our observations. However, this technique was less sensitive in detecting IL-1.

In normal skin, both IL-1 alpha and IL-1 beta were present in all samples from the twelve normal individuals, with prominent reactivity for IL-1 alpha (Fig 1*A*, *B*). The strongest antibody reaction was localized to the stratum corneum, and a lesser amount was detected in the squamous layer. In nine of our normal specimens, the pattern was predominantly intercellular (Fig 1*A*), while three specimens demonstrated both intracellular cytoplasmic and intercellular localization (Table II). In three cases immunoreactive IL-1 was present within the basal cell layer.

IL-1 Alpha and IL-1 Beta in Psoriatic Skin As in the normal individuals, the skin from patients with psoriasis stained positively for IL-1 alpha and/or IL-1 beta, with more intense staining for IL-1

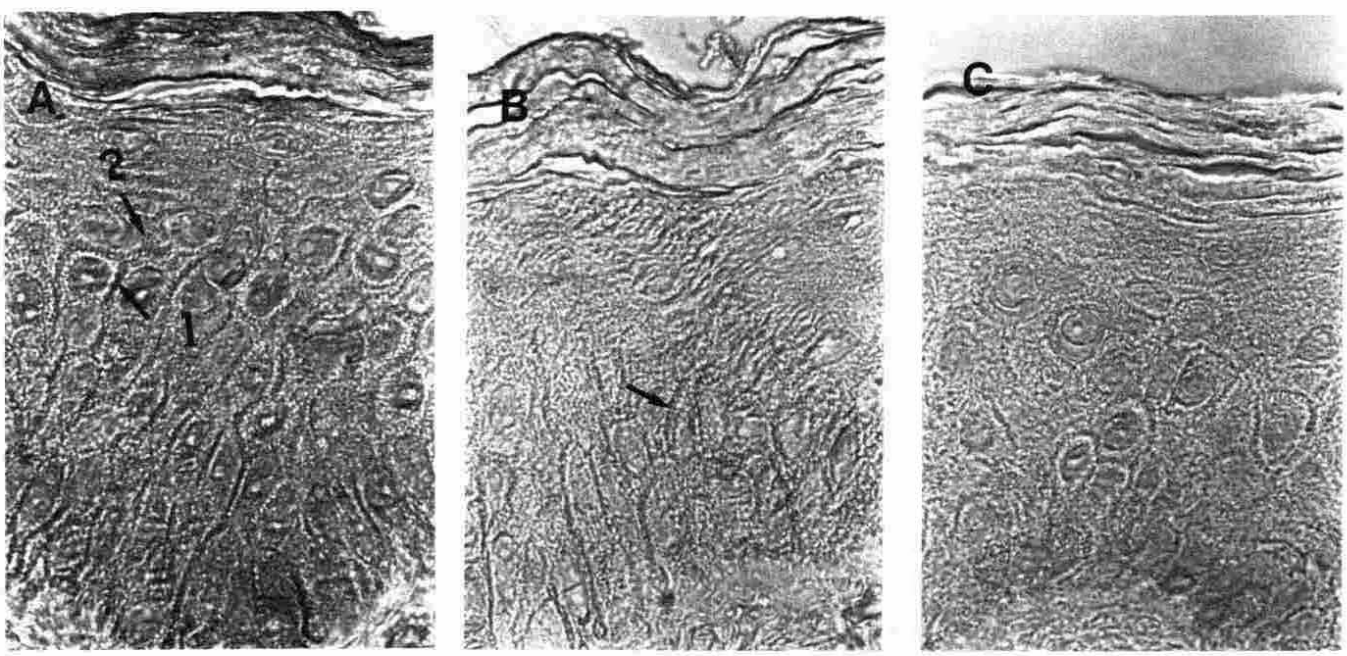


Figure 1. Immunoenzymatic localization of IL-1 alpha *(A)* and IL-1 beta *(B)* in normal skin. Two patterns of staining are present: 1. an intercellular pattern with strong reactivity around the cell membrane and 2. a cytoplasmic pattern which is less prominent. IL-1 alpha is clearly present in the stratum corneum. Some intercellular immunoreactivity for IL-1 beta *(B, arrow)* is also present. The IL-1 beta immunostaining is not greatly above the background as compared with the reactivity of the irrelevant rabbit IgG used as control *(C)* (1200X).

Table II. Distribution Patterns of IL-1 in Normal and Psoriatic Skin[a]

Tissue	Number of Specimens	Intercellular Only	Cytoplasmic Distribution Only	Both Patterns	Negative
		Immunoreactive IL-1 alpha			
Normal	12	9	0	3	0
Psoriatic	12	0	0	12	0
		Immunoreactive IL-1 beta			
Normal	12	12	0	0	0
Psoriatic	12	0	0	10	2

[a] Different distribution patterns of IL-1 alpha and IL-1 beta in normal and psoriatic samples detected by the avidin-biotin-peroxidase technique, as described in *Materials and Methods*. Only results employing 1/100 dilution of antibody are displayed. The patterns are as illustrated in Figs 1 and 2.

alpha (Fig 2*A*, *B*). However, in contrast to normal skin, IL-1 was consistently present with intense staining both in the squamous layer and basal cell layer. Though some specimens (six of 12) demonstrated IL-1 alpha and IL-1 beta in the stratum corneum, the staining was less intense, suggesting decreased amounts of immunoreactive IL-1 alpha and IL-1 beta in psoriatic stratum corneum. It should be noted that although the magnification is the same for both normal and psoriatic skin, the increased size of the psoriatic keratinocytes make them appear larger [25]. Similarly, the marked acanthosis make it impossible to visualize all layers of the epidermis in the same high-power microscopic field. Thus, the photomicrographs are equivalent.

The cellular distribution of IL-1 alpha and IL-1 beta also differed in psoriatic skin. All the psoriatic specimens had a prominent intracellular cytoplasmic pattern as well as a intercellular staining (Fig 2*A*, *B*). In two cases, no staining was present even at the higher concentration of anti-IL-1 beta (1/100 dilution). These two specimens were negative for this cytokine in two different experiments with duplicate samples (Table II); excluding technical reasons as the explanation for failure to detect IL-1 beta.

Specificity Controls In order to confirm the specificity of the reaction, an inhibition assay was developed. Polyclonal IL-1 alpha and IL-1 beta antibodies were premixed with recombinant IL-1 alpha and IL-1 beta, respectively, before incubation with the tissue sections. We found specific inhibition of the reactivity in a dose-dependent fashion. No epidermal staining was observed when 5 μg/ml of rIL-1 alpha was preincubated with 2.5 μg/ml of anti-IL-1 alpha prior to treatment of tissues (Fig 3*A*, *B*). No immunostaining was present when 80 μg/ml of rIL-1 beta was mixed with 5 μg/ml of anti-IL-1 beta (Fig 4*A*, *B*). However, this immunoreactivity was not blocked when the same anti-IL-1 beta concentration was preincubated with serial dilutions of rIL-1 alpha (concentration 80 to 5 μg/ml) (Table III). The incubation of normal and psoriatic samples with the irrelevant rabbit IgG was consistently negative, confirming the specificity of the reaction.

DISCUSSION

A wide variety of inflammatory diseases are characterized by infiltration of polymorphonuclear and mononuclear cells into the skin. However, the inducers and modulators of these inflammatory processes are not well understood. It has been proposed that the keratinocytes participate in this immune and inflammatory response by elaborating a factor indistinguishable biochemically and functionally from the macrophage derived IL-1 [8–12]. This cytokine can be detected in large amounts in stratum corneum from normal skin and has an autocrine proliferative effect on keratinocyte cultures [15,17,18]. Because psoriasis is characterized by epidermal hyperproliferation and inflammatory cell infiltration, we compared the presence of IL-1 in psoriatic and normal skin.

In order to study in situ localization of IL-1 alpha and IL-1 beta in epidermis, we used the immunoenzymatic avidin-biotin-peroxidase technique [23,24], a highly sensitive method to localize antigens in

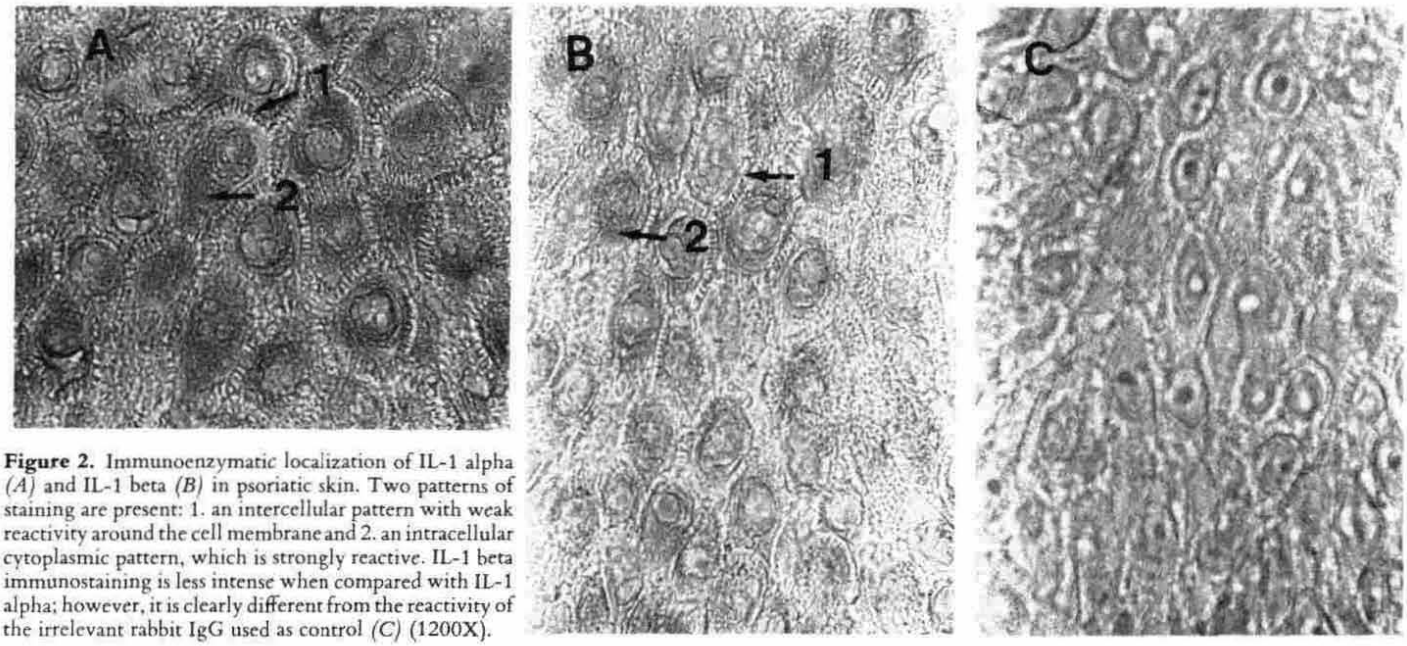
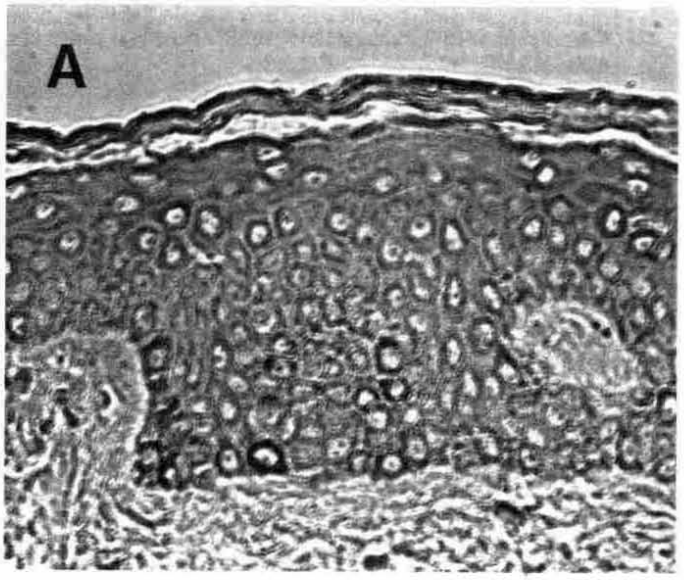


Figure 2. Immunoenzymatic localization of IL-1 alpha *(A)* and IL-1 beta *(B)* in psoriatic skin. Two patterns of staining are present: 1. an intercellular pattern with weak reactivity around the cell membrane and 2. an intracellular cytoplasmic pattern, which is strongly reactive. IL-1 beta immunostaining is less intense when compared with IL-1 alpha; however, it is clearly different from the reactivity of the irrelevant rabbit IgG used as control *(C)* (1200X).



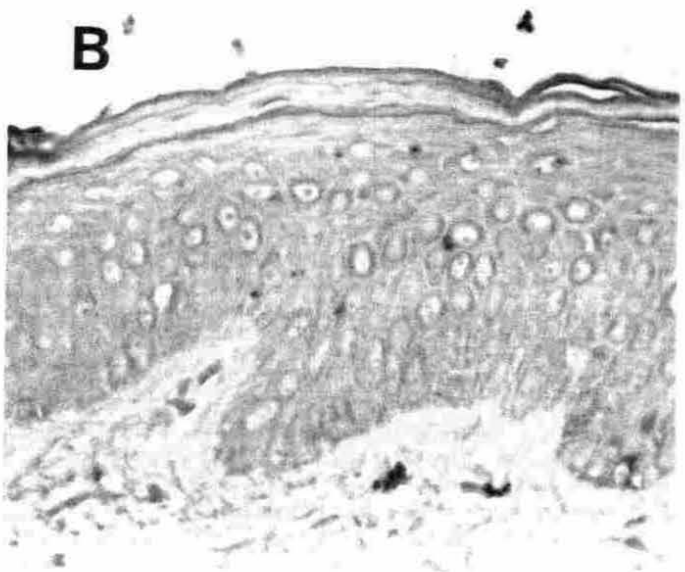
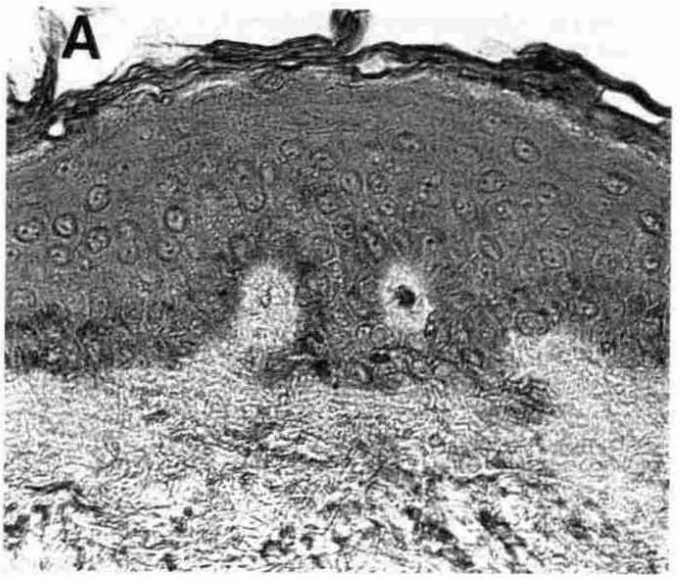


Figure 3. Blocking studies for IL-1 alpha. Positive immunoreactivity for IL-1 alpha is present in *A*. Blocking effect by prior antigen-antibody absorption, as described in *Materials and Methods,* is illustrated by the absence of immunostaining *(B)* (500X).



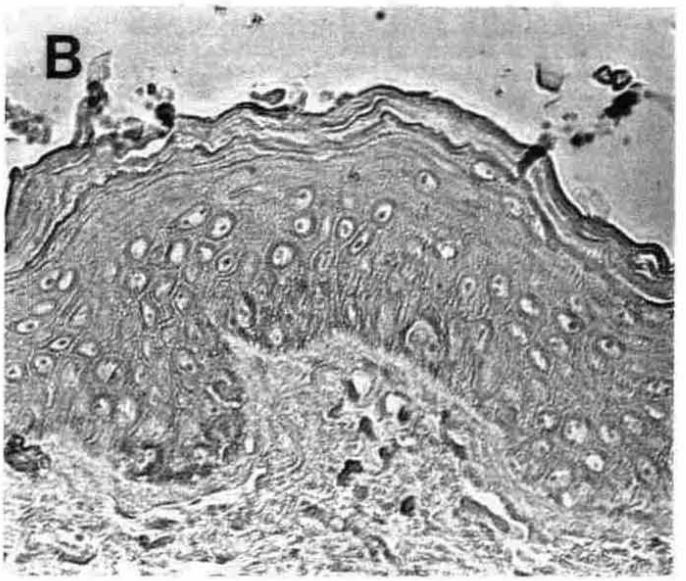


Figure 4. Blocking studies for IL-1 beta. This particular skin section clearly demonstrates immunoreactivity for IL-1 beta *(A)*. Specific blocking effect by antigen-antibody competition, as described in *Materials and Methods,* is illustrated by the absence of immunostaining *(B)* (500X).

formalin fixed tissues. This technique has the advantage of preserving cytologic features so that the nature of the positively stained cells can be precisely evaluated. Using monospecific polyclonal antibodies, we clearly demonstrated both cytoplasmic and intercellular localization of IL-1 alpha. The intense IL-1 alpha reactivity at the intercellular border is consistent with the work of Goldminz et al [26]. Their studies indicated that this cytokine is membrane associated, as demonstrated by significant IL-1 activity in cell membrane preparations derived from human foreskin epidermal cultures. Additionally, Oxholm et al [27] found both intercellular and IL-1 cytoplasmic immunostaining for IL-1 in normal skin; however, high concentrations of antibody were required for detection of cytoplasmic IL-1. Our studies of IL-1 alpha in normal skin are consistent with these reports because both intercellular and cytoplasmic IL-1 alpha were detected. Studies of in situ hybridization in normal murine epidermis demonstrating only mRNA for IL-1 alpha [14] are also consistent with our findings of greater amounts of IL-1 alpha.

Our findings also suggest that IL-1 beta is present in low amounts. Previous reports did not distinguish IL-1 alpha and IL-1 beta by immunostaining [27]. High concentrations of antibody were required in our studies to detect IL-1 beta. Staining was not greatly above background, and careful attention to technical detail was necessary for reproducibility. If methanol treatment was omitted, resulting in a higher background, IL-1 beta activity could not be distinguished. However, the blocking studies confirm the specificity of our antibodies. Previous demonstrations of small amounts of mRNA for IL-1 beta in normal skin support our findings [11]. An additional explanation for differences in our results may be related to affinity of the antibodies employed for different epitopes of IL-1 beta.

IL-1 alpha was also the predominant cytokine in psoriatic skin samples. However, the principal site was cytoplasmic. In contrast to the normal skin, at least half of the psoriatic specimens lack IL-1 in the stratum corneum, yet the squamous layer was consistently positive, suggesting a rearrangement of the normal pattern of cytokine

Table III. Blocking Assays by Antigen-Antibody Absorption Illustrate the Specificity of Our Anti-IL-1 Alpha and Anti-IL-1 Beta Antibodies[a]

Antibody Concentration (μg/ml)	Recombinant Interleukin-1 (μg/ml)	Tissue Immunoreactivity
anti-IL-1 alpha	rIL-1 alpha	
2.5	5	negative
2.5	2.5	negative
2.5	1.25	negative
2.5	.6	negative
2.5	.3	+++
Anti-IL-1 beta	rIL-1 beta	
5	80	negative
5	40	+
5	28	+
5	16	++
Anti-IL-1 bea	rIL-1 alpha	
5	80	++++
5	40	+++
5	20	++++
5	10	++++
5	5	++++

[a] The antibody concentration was fixed (as indicated), and decreasing amounts of antigen (rIL-1 alpha or rIL-1 beta) were added. As the antigen concentration was reduced, tissue staining appeared. The immunohistochemical studies were performed as described in *Materials and Methods*. No cross-reactivity between IL-1 alpha and beta was observed.

localization. Interesting, the squamous layer in psoriasis is mainly characterized by increased cellular proliferation and inflammatory cells infiltration, processes that can be modulated by IL-1.

Although the biologic relevance of our findings are unknown, we speculate that the high rate of keratinocyte proliferation and the epidermal neutrophil infiltration in psoriasis might be related to in situ overproduction of IL-1 with subsequent autocrine stimulation and cellular recruitment. In order to further delineate the relationship between IL-1 and disease activity in psoriasis studies correlating the presence of IL-1 with disease activity are in progress.

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REFERENCES

1. Dinarello CA: Interleukin-1. Rev Inf Dis 6:51–95, 1984
2. Dinarello CA: An update of human interleukin-1: From molecular biology to clinical relevance. J Clin Immun 5:287–97, 1985
3. Miossec P, Yu C, Ziff M: Lymphocyte chemotactic activity of human interleukin 1. J Immunol 133:2007–11, 1984
4. Granstein RD, Margolis R, Mizel SB, Sauder DN: In vivo inflammatory activity of epidermal cell-derived thymocyte activating factor and recombinant interleukin-1 in the mouse. J Clin Invest 77:1020–27, 1986
5. Auron PE, Webb AC, Rosenwasser LJ, Mucci SF, Rich A, Wolff SM, Dinarello CA: Nucleotide sequence of human monocyte interleukin 1 precursor cDNA. Proc Natl Acad Sci 81:7907–11, 1984
6. Gubler U, Chua AO, Stern AS, Hellman CP, Vitek MP, Dechaira TM, Benjamin WR, Collier KJ, Dukovich M, Familletti PC, Fiedler-Nagy C, Jenson J, Kaffka K, Kilian PL, Stremlo D, Wittreich BH, Woehle D, Mizel SB, Lomedico PT: Recombinant human Interleukin-1 alpha: Purification and biological characterization. J Immunol 136:2492–97, 1986
7. Kilian PL, Kaffka KL, Stern AS, Woehle D, Benjamin WR, Dechiara TM, Gubler U, Farrar JJ, Mizel SB, Lomedico PT: Interleukin-1 alpha and interleukin-1 beta bind the same receptor on T cells. J Immunol 136:4509–14, 1986
8. Luger TA, Stadler BM, Katz SI, Oppenheim JJ: Epidermal cell (keratinocyte) derived thymocyte-activating factor (ETAF). J Immunol 127:493–98, 1981
9. Sauder DN, Carter CS, Katz SI, Oppenheim JJ: Epidermal cell production of thymocyte activating factor (ETAF): J Invest Dermatol 79:34–39, 1982
10. Luger TA: Possible relevance to dermatology of epidermal cell thymocyte-activating factor (ETAF). Am J Dermatol 5:97–102, 1983
11. Kupper TS, Ballard DW, Chua AO, McGuire JS, Flood PM, Horowitz MC, Langdon R, Lightfoot L, Gubler U: Human keratinocytes contain mRNA indistinguishable from monocyte interleukin-1 alpha and beta mRNA: J Exp Med 164:2095–2100, 1986
12. Bell TV, Harley CB, Stetsko D, Sauder DN: Expression of mRNA homologous to interleukin-1 in human epidermal cells. J Invest Dermatol 88:375–79, 1987
13. Sauder DN, Arsenault TV, Stetsko D, Horley CB: Isolation and partial characterization of a putative ETAF. cDNA clone (k IL-1): ETAF is distinct from IL-1 alpha or IL-1 beta. J Invest Dermatol 88:515A, 1987
14. Takacs L, Kovacs EJ, Smith MR, Young HA, Durum SK: Detection of IL-1 alpha and IL-1 beta gene expression by "in situ" hybridization. J Immunol 141:3081–3089, 1988
15. Gahring LC, Buckley A, Daynes RA: Presence of epidermal-derived thymocyte activating factor/interleukin-1 in normal human stratum corneum. J Clin Invest 76:1585–91, 1985
16. Hauser C, Saurat J, Schmitt A, Jaunin F, Dayer J: Interleukin-1 is present in normal human epidermis: J Immunol 136:3317–23, 1986
17. Sauder DN: Biologic properties of epidermal cell thymocyte-activating factor. J Invest Dermatol 85:1765–825, 1985
18. Gilchrist BA, Sauder DN: Autocrine growth stimulation of human keratinocytes by epidermal cell-derived thymocyte activating factor (ETAF): Implications for cellular aging. Clinical Research 32:585A, 1984
19. Sauder DN, Monick MM, Hunninghake GW: Epidermal cell-derived thymocyte activating factor (ETAF) is a potent T-cell chemoattractant: J Invest Dermatol 85:431–433, 1985
20. Camp RDR, Fincham NJ, Cunningham FM, Greaves MW, Morris J, Chu A: Psoriatic skin lesions contain biologically active amounts of an interleukin-1 like compound. J Immunol 137:3469–74, 1986
21. Konnikov N, Ree HJ, Dinarello CA, Pincus SH: Interleukin-1 (IL-1) is present in psoriatic plaques. J Invest Dermatol 88:499A, 1987
22. Dinarello CA, Ikejima T, Warner SJC, Orencole SF, Lonnemann G, Cannon JG, Libby P: Interleukin-1 induces interleukin-1: J Immunol 139:1902–10, 1987
23. Hsu S-M, Raine L, Fanger H: Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: A comparison between ABC and unlabeled antibody (PAP) procedures. J Histo Cyto 29:577–80, 1981
24. Hsu S-M, Zhao X: Localization of interleukin-1 in normal or reactive lymphatic tissue and skin: Abundance of IL-1 in interdigitating reticulum cells. Lymph Rel 6:13–8, 1987
25. Mali JWH: Psoriasis: A dynamic disease. Br J Dermatol 101:725–730, 1979
26. Goldminz D, Kupper TS, McGuire TS: Keratinocyte membrane associated epidermal cell-derived thymocyte-activating factor (ETAF). J Invest Dermatol 88:97–100, 1987
27. Oxholm A, Oxholm P, Staberg B, Bendtzen K: Immunohistological detection of Interleukin 1-like molecules and tumour necrosis factor in human epidermis before and after UVB-irradiation "in vitro". Br J Dermatol 118:369–76, 1988